COMPARISON OF GRAZING AND SUBSTRATE TYPE ON PERIPHYTON GROWTH

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CHAPTER 1

INTRODUCTION

Water quality is impaired in many streams and rivers of the United States. The primary source of the pollution is from non-point sources including nutrient, sediment, and bacteria loading (U. S. EPA, 1992). Although water quality has improved since the enactment of the Clean Water Act of 1972 (U. S. EPA, 1987), the U. S. Environmental Protection Agency reported non-point source pollution as the primary source of pollution of our Nations waters. Increases in nutrient loading associated with human activity have resulted in an increase of eutrophic conditions for many lotic ecosystems (Evenson et al., 1981). The traditional approach to water quality management within watershed studies has been to monitor the chemical and physical parameters to assess the status of water quality within the watershed.

The goal of the Clean Water Act is to protect the chemical, physical, and biological integrity of our Nation's water. Monitoring only chemical and physical conditions may not provide adequate information on the status of the biological conditions of the streams and rivers. Biological communities within a lotic ecosystem represent the ecological integrity including chemical, physical, and biological properties.

Bioindicators are useful for detecting and/or assessing the impairments of aquatic life within a lotic ecosystem. Periphyton assemblages (algae) are useful

for water quality monitoring. Periphyton represents the primary producer trophic level and is responsive to both chemical and physical factors. Primary producers provide the bases of the food web within a lotic ecosystem. Primary productivity is defined as the rate of formation of organic matter from inorganic carbon through photosynthesis (Hauer and Lamberti, 1996). Chlorophyll a measurements are used as an indicator of primary productivity. Chlorophyll a concentration provides an estimate of algal bio-mass and may be collected over time at designated sample sites and compared. High chlorophyll a concentrations may indicate nutrient enrichment and low chlorophyll a concentrations may indicate low nutrient availability, toxic conditions, or light limiting conditions. By combining the algal data with macro-invertebrate and fish data, the process of biological assessment is significantly enhanced (U. S. EPA, 1997).

The Matlock Periphytometer was developed for the purpose of assessing water quality (Matlock et al., 1998). The Matlock Periphytometer is a quantitative, passive diffusion periphyton nutrient enrichment system, which measures the primary productivity of a lotic ecosystem, determines the limiting nutrients at a given site over a given time, determines the streams response to increased levels of known nutrients, and determines the maximum potential productivity of a stream. Advantages of the Matlock Periphytometer include a quantifiable nutrient diffusion rate, total extraction of algae from its growth substrate, and it is simple and economical to construct and implement. The Matlock Periphytometer

utilizes artificial substrate and may be placed in both wadeable and non-wadeable streams, rivers and reservoirs.

Although all states are encouraged to develop and incorporate biological surveys as part of their water quality management programs, only a few states have developed protocols for assessing periphyton assemblages. The Oklahoma Conservation Commission uses glass rod periphytometer for collecting periphyton assemblages. The periphyton is allowed to colonize on the glass rod substrate over a two-week period of time. The rods are then retrieved and the periphyton carefully extracted and analyzed for determination of chlorophyll a and/or taxa. Comparing historical chlorophyll a data using the glass rod periphytometer to current data using the Matlock Periphytometer requires assessing the comparability of methods.

There is evidence that grazing may effect measures of periphyton growth on artificial substrates (Fuller et al, 1998; Gelwick et al., 1997; Holomuzki, 1998; Kuferberg, 1997). Grazing may be so significant as to limit the available algae bio-mass. The interaction between light, flow, and grazing may impact the composition and growth of algal bio-mass. Substrate configuration may also influence periphyton growth on artificial substrates. Historically, research has been conducted on the Illinois River Basin in eastern Oklahoma, U. S. A. using periphyton as a measure of water quality (Matlock et al., 1999a; Oklahoma Conservation Commission, 1993). As new procedures and methodologies for

assessing periphyton growth and responses are developed and implemented for collecting periphyton data, the ability of water quality managers to correlate both historical data collected by existing methodologies and data collected by new methodologies is necessary to assure proper use of the data for assessing water quality.

Research Objectives

The objective of this research is to determine if productivity estimates from a glass rod periphytometer are comparable to productivity estimates from a Matlock Periphytometer. This research was designed specifically to:

1. Compare chlorophyll *a* data from glass rod periphytometers within a protective screen to Matlock Periphytometers chlorophyll *a* data within a protective screen;
2. Compare chlorophyll *a* data from glass rod and Matlock Periphytometers within a protective screen to chlorophyll *a* data from glass rod periphytometers without protective screening.

This thesis represents the research conducted to accomplish these objectives.

CHAPTER 2

LITERATURE REVIEW

Periphyton as Indicator of Trophic Status

The periphyton assemblage is a useful component of bio-assessment for water quality monitoring. Many state and federal agencies have adopted protocols for assessing aquatic habitat, macro-invertebrates, and fish (U. S. EPA, 1997) as part of an integrated physical, chemical, and biological assessment of the Nation's water quality. Benthic algae (periphyton) samples may be collected to develop a list of taxa present in the sampling reach, or to measure algal community structure within selected habitats (Dodds et al., 1998).

Only a few state and federal agencies have incorporated periphyton assemblages into their water quality monitoring program (U. S. EPA, 1997). Periphytic algae represent the primary producer in lower order streams and are responsive to a range of environmental variables. The structural/composite analyses of periphyton may be focused upon either taxonomic or non-taxonomic measurements. The methodology for periphytic algal bio-indicator research has been reviewed with emphasis on needs for current and future research direction (Robinson, 1983). The methods used for quantifying the interaction among periphytic community components and regulation of the effecting environmental parameters must be stressed (Robinson, 1983). The dynamics of periphyton communities are representative of the long-term

maturation of aquatic systems, or eutrophication (Roos, 1983). Annual fluctuations among the periphytic communities are brought about by many factors including availability of nutrients, toxins, light, grazing, or substrata.

Effects of Nutrients

The effects of nutrient enrichment upon shallow water ecosystems are much more complex than deeper lakes that have been the central focus of eutrophication investigations (Hann et al., 1997). Both the diversity of primary producers that compete for nutrients and the proximity of sediments that bind nutrients contribute to the complexity in the role of nutrient loading on the shallow water ecosystems (Hann et al., 1997).

Nutrient loading attributed to human activity has been accelerated in many aquatic ecosystems (U. S. EPA, 1990). The resulting increase in primary productivity can result in nuisance level of algal growth, eutrophication, and aquatic habitat loss (Patrick, 1977; Hill et al., 1992). Periphyton growth in a stream is limited by availability of resources such as light, nutrients and space (Sand-Jensen, 1983). Measuring the response of a primary producer community to direct nutrient enrichment can provide a reliable indication of nutrient limitation for a specific community (Matlock et al., 1998; Matlock et al., 1999b). A particular nutrient may limit periphytic growth until it becomes available in concentrations greater than utilized by the algae, or another nutrient concentration falls below that threshold level and becomes the limiting nutrient.

A study on tadpole effect on nutrients suggest that grazing tadpoles did not affect nutrient (NO_3 and PO_4) concentrations in enclosed systems (pond) probably because ambient concentrations were beyond growth-limiting levels (Holomuzki, 1998).

Effects of Light

A review of studies showing the effects of light on periphyton growth suggest that light may be limiting only in heavily shaded water bodies, where leaf canopies can intercept more than 95% of the incident radiation (Hill et al., 1995). Light may not be a factor in nutrient-replete conditions (Van Dijik, 1993). Light may be instrumental in establishing periphytic community structure and in modifying the impact that grazers have on algae (Wellnitz et al., 1996).

Effects of Grazing

A review of literature investigating the affects of periphyton grazers shows positive removal rates of algal bio-mass (Cattaneo and Roberge, 1991). Determining the effects of grazing by herbivores is complicated by the interaction of environmental factors, including the composition of species and the bio-mass of the total periphytic community (Kupferberg, 1997). Kupferberg (1997) examined how interactions between resources that vary in edibility and herbivores that vary in ability to acquire resources, control primary productivity in a northern California river. The study used cobbles containing a relatively inedible filamentous green alga (*Cladophora glomerata*), and more nutritious

epiphytic diatoms. These algae covered cobbles that were exposed or excluded from grazing by two species of tadpoles (*Rana boylii* or *Hyla regilla*). The results of the study indicate the effects of grazing by *Rana boylii* tadpoles decreased the abundance of diatoms and detritus on Cladophora, but resulted in an increase in total periphyton bio-mass and area-specific primary productivity on cobbles. *Hyla regilla* is a much smaller and less efficient scraper than its *Rana* counterpart and did not significantly reduce Cladophora abundance relative to controls, which may indicate Cladophora growth (Kupferberg, 1997).

For algae, tradeoffs between grazing resistant structures and competitive ability of algae have been shown to cause inedible taxa to increase in abundance but not to increase total periphyton bio-mass (Rosemond, 1993). A study of the effects of grazing by the green frog tadpoles (*Rana clamitans*) in a fish-less woodland pond in central Kentucky has shown that tadpoles can significantly reduce benthic algae bio-mass in light levels that mimic springtime canopy conditions (Holomuzki, 1998). Tadpoles had little effect on algal species composition. The study suggests both tadpoles and light affect the accrual of the algae bio-mass. Nutrient levels in the pond very likely exceeded growth-limiting levels and were more likely to determine the composition of algae assemblages in the pond than were the tadpoles (Holomuzki, 1998). In ambient densities, green frog tadpoles can significantly reduce benthic bio-mass. Studies have reported that grazing by larval amphibians can affect the phytobenthos in lentic

(Bronmark et al., 1991) or lotic (Lamberti et al. 1992; Holomuzki and Hemphill, 1996) systems.

Grazing effects by tadpoles tend to be patchy and temporally limited. Tadpoles often aggregate in large numbers in warmer, shallow areas of ponds and streams, resulting in localized grazing. In addition, tadpoles are typically only temporary inhabitants of aquatic systems and the impact will depend on their duration in the system (Duellman and Trueb, 1986).

Current theory is that fish primarily influence periphyton communities by controlling herbivore densities. A study of the interaction of the molluscivorous red-ear sunfish (*Lepomis microlophus*), and snails (*Physella heterostropha*) in a replicated factorial experiment determined that although fish only had visual and waterborne (olfactory) contact with the snails, their presence inhibited snail reproduction and/or increased mortality of small snails. Twice as many snails were produced in the absence of fish than in the presence of fish (McCollum et al., 1998). The study showed that snail grazing was six times higher in the absence of fish and reduced periphyton cell number and increased the average size of the periphyton cells, primarily through effects on green algae. Snails also reduced the bio-volume of diatoms and blue-green algae.

A study on the effects of grazing minnows (*Campostoma*) on spatial and temporal heterogeneity of the vertical height of periphyton was measured in an artificial stream and Brier Creek in eastern Oklahoma (Gelwick and Matthews,

1997). The study showed that grazing by the minnows reduced spatial and temporal heterogeneity of algae height in the artificial stream, but not in the pools of natural streams. Grazing minnows avoid water less than 20 cm deep (Power et al., 1985) due to predation from wading birds (Harvey and Stewart, 1991). This could have resulted in the increased heterogeneity as the algae in the shallow areas remained ungrazed (Gelwick and Matthews, 1997).

A study determining the effects of mayfly (*Rhithrogena robusta*) grazing in a subalpine stream in central Colorado had shown significant reduction of algal bio-volume under all light regimes, although species assemblages differed between light treatments (Wellnitz et al., 1996). Another study was conducted using the mayfly *Stenonema sp.* and caddisfly *Psilotreta sp.* in New York in both an artificial stream and a natural stream. Although significant reduction of algae bio-mass was determined under the controlled laboratory setting, there was no significant reduction in bio-mass or chlorophyll a concentration among grazer density treatments in the natural stream (Fuller et al., 1998). The results suggest that the laboratory stream chambers may have influenced biotic (predation/composition) and/or abiotic (flow regime, etc.) factors.

Effects of Substrata

Periphytic algae are primary producers and are sensitive indicators of environmental changes within a lotic ecosystem (Dodds et al., 1998; Matlock et al., 1998; Matlock et al., 1999a; Matlock et al., 1999b). As periphytic

assemblages are attached to the substrate, they integrate the effects of physical and chemical perturbation to the stream. Periphyton is characterized as having high species number and a rapid response time to exposure, thus making it a good biological indicator. Artificial substrata have been successfully used for many types of periphytic investigations (rates of colonization, community interactions, impact and comparisons of environmental variability and collection of algae). Artificial substrate should not be used in situations where they are intended to mimic natural substrates (Robinson, 1983). Periphyton taxa can be identified to species and assessed for tolerance or sensitivity to changes in environmental conditions (Dixit et al., 1992). Kentucky Department of Environmental Protection has developed its protocol for periphyton assemblages (U. S. EPA, 1997). The Oklahoma Conservation Commission has developed its protocol for collection of periphyton assemblages (U. S. EPA, 1997). Oklahoma currently uses artificial (glass rod) substrates.

Non-taxonomic measurements of periphyton are useful in determining ecosystem functions such as primary productivity, growth rate, and/or bio-mass accumulation. The use of periphyton bio-mass as a water quality indicator has been limited because of the problem of reproducibility between replicates (Wetzel, 1983). Variations between replicates are attributed to the irregularity of periphytic growth on the substrata, grazing pressure, and the errors associated with sample collection and analyses. The patchiness of periphytic growth can be

resolved by sub-sampling a larger substratum area using consistent artificial substrates (Dilks and Meier, 1981).

Glass tubes or rods have been tested as artificial periphyton substrata (Meier et al., 1983). The study showed the variability in chlorophyll a concentration from replicates using natural substrate were higher than 25% as compared to a variability of less than 4% using the artificial substrate. The use of artificial substrate resulted in consistent chlorophyll a data collection, and in a considerable amount of time being saved by expediting the field sampling activities and performing much of the sample preparation in the controlled environment of a laboratory.

Teflon strips have been used as an artificial substrate to predict phytoplankton response to nutrient enrichment (Smoot et al., 1998). The advantages of the approach in the study were: (1) it uses well established protocols for chlorophyll a analyses; (2) the artificial substrate provide a durable, consistent, workable, and simple inert surface; (3) the substrate and its associated biochemical methods are time and cost competitive and allow for an experimental design involving scores of analyses (Smoot et al., 1998).

Klapwijk et al. (1983) used a modified artificial substrate method to study the effects of agricultural pollutants on benthic algae in ditches that drained wetlands in the Netherlands. The study used glass microscope slides suspended from

floating plexi-glass racks. After a two-week exposure time, the slides were collected and analyzed for both benthic bio-mass and species composition. Although successful, much attention was given to the potential effects light limitations due to the floating algae and duckweed (Klapwijk et al., 1983).

Matlock et al. (1998) developed a quantitative passive diffusion method (Matlock Periphytometer) for measuring in situ the periphytic community response to nutrient enrichment. The Matlock Periphytometer is constructed of a nylon membrane bio-filter (0.45μm), a glass fiber filter, and a one-liter (L) low-density polyethylene flexible nutrient reservoir. The bio-filter allows for quiescent diffusion of nutrients from the bottle through the glass fiber filter, which serves as a growth substrate. Nutrient movement through the membrane and filter is driven by passive diffusion, and is predictable under Flick's Law (Matlock et al., 1998)

The Catherwood Diatometer is a floating rack of cleaned microscope slides used extensively by the Pennsylvania Academy of Natural Sciences for collection of diatoms assemblages for biological monitoring of water quality in areas of waste water discharge into receiving waters (Friant and Koerner, 1980). The procedure has been successfully used *in- situ* for monitoring select trace metals and other algal responses to pollutants.

CHAPTER 3

COMPARISON OF GRAZING AND SUBSTRATE TYPE ON PERIPHYTON GROWTH

Introduction

The use of periphyton assemblages as part of the biological assessment process can be an effective measure for water quality assessment (U. S. EPA, 1997). The use of artificial substrate for colonizing and sampling periphyton provides a consistent and workable surface which reduces the variability associated with sampling from natural substrates (Smoot et al., 1998). As discussed in the previous chapter, there are many environmental influences associated with any method measuring periphyton growth. Grazing, flow, light, and water depth all may effect periphyton growth (Wellnitz et al., 1996).

The Matlock Periphytometer was developed as a method for measuring the periphytic community response to nutrient enrichment in a stream using passive diffusion through a semi-permeable membrane and glass fiber filter (Matlock et al., 1998). The glass rod periphytometer consists of two solid glass rods of consistent length and diameter mounted on a wire hanger (Oklahoma Conservation Commission, 1993). Two types of periphytometers were deployed together under various control regimes. This investigation evaluates the effects of grazing and growth substrate on periphyton growth using two types of periphytometers placed in three tributaries (Battle Creek, Tyner Creek, and

Peacheater Creek) of the Upper Illinois River Basin in eastern Oklahoma (Figure 1). Battle Creek sample site was located at 94°41'30" latitude, 35°57'15" longitude; Peacheater Creek sample site was located at 94°41'15" latitude, 35°57'15" longitude; and Tyner Creek sample site was located at 94°43'30" latitude and 36°1'45" longitude.

The Upper Illinois River Basin is located in northeast Oklahoma and northwest Arkansas and consists of approximately 400,000 hectares. All three watersheds are similar in topography and land use with an average annual rainfall of 110 cm. Land use is predominantly pasture and woodland with a significant number of poultry producers. Historical water quality data have been compiled by U. S. Geological Survey Water Resource Data from years 1991 through 1994 (USGS, 1991 – 1994), and from non-published data from the Oklahoma Conservation Commission (Table 1). Mean concentrations of Nitrate-Nitrite nitrogen range from 1.98 to 2.27 mg/l between each of the three sample sites. The mean concentration of Ammonia Nitrogen is 0.02 mg/l for both Peacheater creek and Battle Creek. Ammonia Nitrogen data for Tyner creek were not available. Mean concentrations of Total Phosphorus were highest for Battle Creek at a mean concentration of 0.13 mg/l as compared with 0.07 and 0.02 mg/l for Peacheater Creek and Tyner Creek, respectively. Battle Creek Watershed covers less than half the area as compared to Peacheater Creek and Tyner Creek watersheds but contained the highest level of nutrients.

Methods

The Matlock Periphytometer is a passive diffusion nutrient enrichment apparatus which is constructed of a cellulose semi-permeable dialyses membrane (Spectra-Por® 08-667B-25 mm, 12 to 14 kilodalton (kD) pore size, Spectrum Medical Industries, Inc., Los Angeles, California), a glass fiber filter (Whatman® 934-AH, 37 mm, 1.5 μm pore size, Whatman International Limited, Maidstone, England), and a one liter low-density polyethylene flexible nutrient reservoir (Cubitainer®, Texberry Container Corp., Houston, Texas) (Figure 2). The dialyses membrane serves as a bio-filter, and the glass fiber filter serves as a growth substrate for the periphyton. Nutrient solutions generally consist of sodium salts, which provided the most biologically available of the targeted nutrients without interference from other nutrients such as calcium and potassium. Concentrations of $NaNO_3$, and Na_2HPO_4-$7H_2O$ solutions were put into solution using de-ionized water to make nitrate, phosphate, and nitrate plus phosphate nutrient solutions. De-ionized water using no nutrients was used as the control.

Each Matlock Periphytometer sampling unit was constructed by filling the container with the nutrient solution, cutting a 2.9 cm diameter hole in the container cap, slicing one side of a hydrated dialyses membrane tubing of 4 cm length (making a 4 cm square), placing the membrane over a filled container mouth, placing a glass fiber filter directly over the membrane, and carefully placing the cap onto the container (Figure 2). Six replicates of each Matlock

Periphytometer treatment was placed onto a rigid aluminum framed rack (Figure 3).

The objective of this investigation was to compare the periphytic assemblage on the control surface of the Matlock Periphytometer and the glass rod periphytometer. The control consisted of de-ionized water, which contains no nutrients. The glass rod periphytometer consisted of a solid glass rod, which was approximately 8 mm in diameter and approximately 67 mm in length. A series of two glass rods were secured to a wire hanger using epoxy resin.

Glass rod periphytometers were randomly placed on the six racks containing the Matlock Periphytometers at each site. Each rack was covered by eight-mesh aluminum wire screen to protect the substrates (periphyton growth surface) from benthic macro-invertebrate and fish grazing. Three sets of two glass rods were placed on the inside of this wire screen. Three sets of two glass rod periphytometers were randomly placed outside the protective screening on three of the six racks. The racks containing the Matlock Periphytometers and the glass rod periphytometers were placed in the stream perpendicular to the stream flow (Figure 4). The racks were placed in an approximately 0.3m deep section of the stream run and anchored to the stream substrate by steel rod driven into the streambed.

The periphytometers were deployed in three tributaries in the Upper Illinois River Basin following the same protocol. The periphytometers were left in the stream for 14 days (April 8 – 21, 1995). At the end of the 14-day growth period, the colonized glass rods and filters were collected, placed into vials containing 3 ml of 90 percent acetone solution saturated with magnesium carbonate, wrapped in aluminum foil, kept at 5° C, and transported to the laboratory for chlorophyll *a* analyses using U. S. EPA Standard Method 10200H3 (APHA, 1989). The chlorophyll *a* collected from the Matlock Periphytometers, and grass rod periphytometers were expressed as $\mu g/cm^{-2}$ by dividing the bio-mass of the extracted chlorophyll *a* by the algae growth surface area of the substrate. The growth area of the Matlock Periphytometer growth substrate was a 29 mm diameter circle with an area of 6.6 cm^2. The growth area of the glass rod periphytometer was approximately 8 mm in diameter and 67 mm in length with an area of 17 cm^2.

Chlorophyll *a* data from all three monitoring sites were pooled in accordance to treatment type. Sample population from each treatment type was tested for homogeneity within each treatment type and between treatment types. Analyses of Variance using the Least Square Means procedure of the SAS System (SAS Institute, Inc., 1990) was used to determine homogeneity within the treatments. The Least Square Difference (LSD) t-test was used to determine if the chlorophyll *a* means between treatments were significantly different at $\alpha = 0.05$ (Steel and Torrie, 1980). The null hypothesis tested was that no significant

difference existed between chlorophyll *a* extracted from the Matlock Periphytometers protected from grazing, glass rod periphytometers protected from grazing, and glass rod periphytometers not protected from grazing.

Results

Chlorophyll *a* was extracted from each sample (Table 2). Two replicates from the Tyner Creek inside glass rod periphytometer (GRP-In) treatment were excluded from analysis due to excess filamentous algal bio-mass accumulation depositing on the filter and not grown on the filter. The Analyses of Variance Least Square Mean test determined homogeneity (α=0.05) among the replicates within each treatment set (Table 3). These results suggest no significant difference within treatments.

The LSD t-test was performed on chlorophyll *a* data from the Matlock Periphytometer, glass rod periphytometer protected from grazing, and the glass rod periphytometer not protected from grazing for Peacheater Creek, Tyner Creek, and Battle Creek, collectively (Table 4). The LSD t-test indicated no significant difference (α =0.05) between chlorophyll a extracted from the Matlock Periphytometer and chlorophyll a extracted from the glass rod periphytometer inside the screened area. However, a significant difference (α=0.05) was indicated between both the Matlock Periphytometer and the glass rod periphytometer outside the screened area, and the glass rod periphytometer inside the screened area and the glass rod periphytometer outside the screened

area (Table 4). Analyses of variance failed to reject the null hypothesis for α=0.05 between the Matlock Periphytometer and the glass rod periphytometer inside the screened area (Table 5). Analyses of variance rejected the null hypothesis for α=0.05 between the Matlock Periphytometer and glass rod periphytometer outside the screened area, and the glass rod periphytometer inside the screened area and the glass rod periphytometer outside the screened area (Table 5).

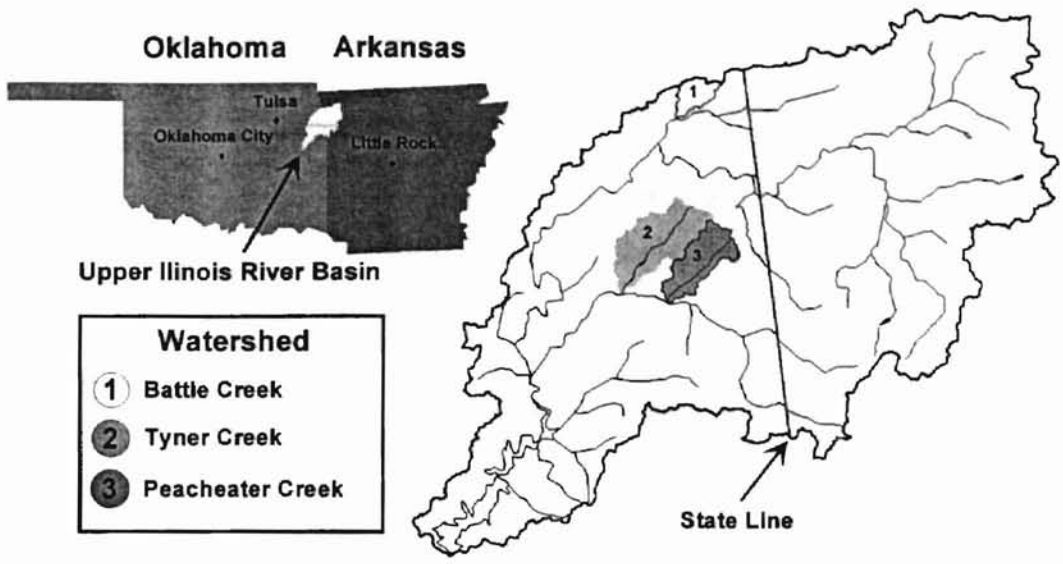

FIGURE 1. Upper Illinois River Basin site location map.

TABLE 1: Historical water quality data from Battle, Tyner, and Peacheater Creeks in the Illinois River Basin in Eastern Oklahoma, expressed as means, minimums (Min), and maximums (Max) (USGS, 1991-1994; OCC, 1995).

Water Quality	Battle Creek 1991-1994			Peacheater Creek 1993			Tyner Creek 1991		
Parameter	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Nitrate-Nitrite Nitrogen (mg l^{-1})	2.16	3.90	0.81	2.27	3.10	1.50	1.98	3.60	0.00
Ammonia Nitrogen (mg l^{-1})	0.02	0.03	0.01	0.02	0.03	0.01	--	--	--
Total Phosphorus (mg l^{-1})	0.13	0.46	0.08	0.07	0.28	0.02	0.04	0.11	0.01
Ortho-Phosphorus (mg l^{-1})	0.11	0.34	0.06	0.05	0.20	0.02	--	--	--

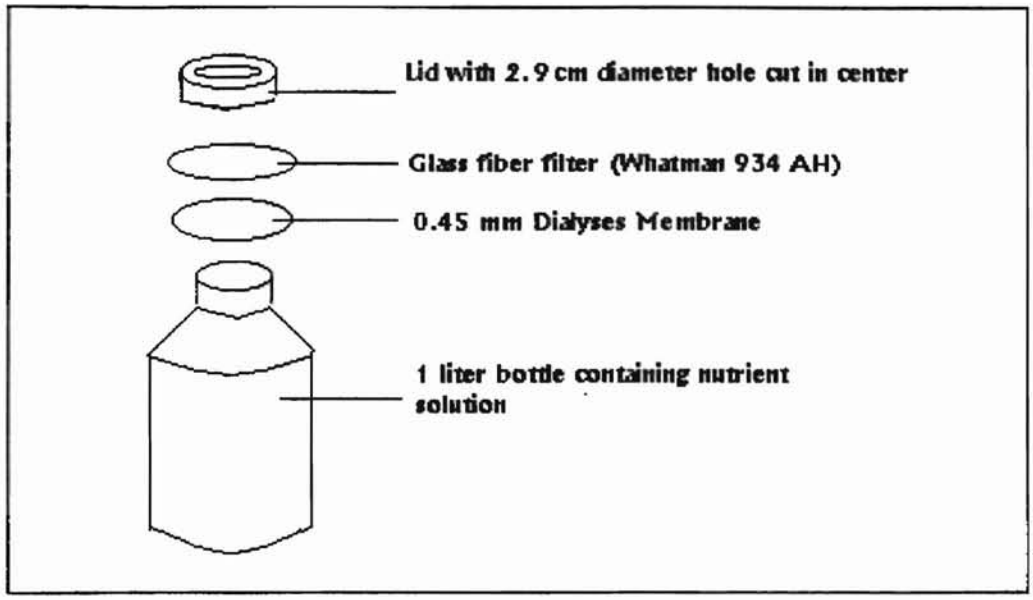

FIGURE 2. Diagram of Matlock Periphytometer.

FIGURE 3. Matlock Periphytometer treatment array.

Discussion

The LSD t-test indicated the mean chlorophyll *a* extracted from the glass rod periphytometer suspended outside the racks was significantly higher than the means of both Matlock Periphytometer and glass rod periphytometer treatments within the protective screening (Figure 5). This could be the result of light limitation by the screen causing a decrease in algae production and/or the select colonization of certain algal species. Another potential cause for the decrease in algal production is inhibited colonization due to aluminum toxicity from the screening. The variance of chlorophyll *a* data from the non-protected glass rod periphytometer was larger than the variance from chlorophyll *a* data from the protected Matlock and glass rod periphytometers (Figure 5). The increase may be attributed to grazing. In addition, grazing may have decreased abundance of more edible algae species, which resulted in an increase in total periphytic bio-mass on the growth substrate through algal tradeoffs. This is consistent with observations of Kupferbuerg (1997).

The LSD t-test did not indicate a significant difference (α=0.05) of chlorophyll *a* data between the Matlock Periphytometer and the glass rod periphytometer suspended inside the screened racks (Table 5). However, an increased mean chlorophyll *a* concentration of the control over the glass rod periphytometer was observed. Robinson (1983) suggested that smoother artificial substrate such as glass result in select colonization of specific algal

FIGURE 4. Matlock Periphytometer deployed in Battle Creek.

TABLE 2: Summary statistics for chlorophyll *a* extracted from Matlock Periphytometers from Peacheater Creek, Battle Creek, and Tyner Creek between April 8-21,1995.

Site	Treatment*	Sample Number (n)	Mean Chlorophyll *a* ($\mu g/cm^2$)	Standard Deviation ($\mu g/cm^2$)
Peacheater Creek	MP-In	6	0.50	0.23
	GRP-In	6	0.39	0.15
	GRP-Out	6	1.08	0.37
Battle Creek	MP-In	6	1.07	0.31
	GRP-In	6	0.19	0.09
	GRP-Out	6	0.51	0.31
Tyner Creek	MP-In	6	0.21	0.14
	GRP-In	4	0.81	0.33
	GRP-Out	6	1.08	0.64

* Matlock Periphytometer in screen (MP-In), glass rod periphytometers in screen (GRP-In), and glass rod periphytometers outside screen (GRP-Out)

TABLE 3: Least Square Means of Matlock Periphytometer in screen (MP-In), glass rod periphytometers in screen (GRP-In), and glass rod periphytometers outside screen (GRP-Out) treatment chlorophyll *a* concentrations ($\mu g\ cm^{-2}$) collected using Matlock Periphytometer at three sample sites from April 8 – 21, 1995.

Treatment	Least Square Mean ($\mu g/cm^2$)	Standard Error ($\mu g/cm^2$)	Degree of Freedom	T Value	Pr>ITI
GRP-In	0.42	0.10	49	3.94	0.0003
GRP-Out	0.88			8.76	0.0001
Control	0.59			5.83	0.0001

TABLE 4: Least Square Difference T test of Matlock Periphytometer in screen (MP-In), glass rod periphytometers in screen (GRP-In), and glass rod periphytometers outside screen (GRP-Out) treatment chlorophyll *a* concentrations ($\mu g\ cm^{-2}$) collected using Matlock Periphytometer at three sample sites from April 8 – 21, 1995.

Treatment Comparison	Difference ($\mu g/cm^2$)	Standard Error ($\mu g/cm^2$)	Degree of Freedom	T Value	Pr>ITI
GRP-In - GRP-Out	-0.46	0.14	49	-3.14	0.002
GRP-In - MP-In	-0.16			-1.13	0.263
GRP-Out and MP-In	0.29			2.07	0.043

TABLE 5: Comparison of treatment means of Matlock Periphytometer in screen (MP-In), glass rod periphytometers in screen (GRP-In), and glass rod periphytometers outside screen (GRP-Out) treatment chlorophyll *a* concentrations ($\mu g\ cm^{-2}$) collected using Matlock Periphytometer at three sample sites from April 8 – 21, 1995.

Treatment	Mean ($\mu g/cm^2$)	Comparison*
MP-In	0.59	A
GRP-In	0.42	A
GRP-Out	0.88	B

*Treatments having the same letter comparisons indicate no significant difference (α=0.05) of chlorophyll *a* concentrations between the treatment means.

species. The porous glass fiber filter substrate provided by the Matlock Periphytometer may generate more representative algal growth response.

Although this research has identified very little differences in periphyton growth response among the treatment, it supports the fact that measuring periphyton is a highly variable system consisting of influences from multiple known and unknown factors.

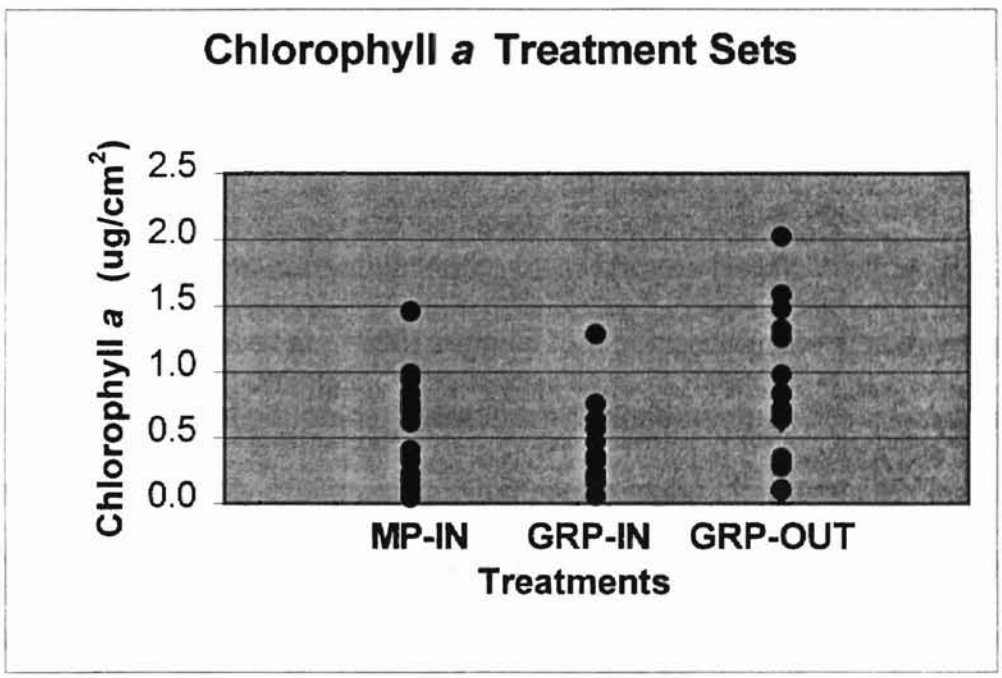

FIGURE 5. Comparison of chlorophyll *a* concentration by treatment.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Conclusion

The use of periphyton as an indicator of water quality within lotic systems is not as widely used as other methods associated with chemical and physical measurements. The results of this research demonstrate the complexity of using periphyton as an indicator of water quality. Environmental effects on periphyton growth vary among geographical locations. Variables which may affect periphyton growth, such as nutrient enrichment, at one site may not exist or be compensated by other variables such as turbidity or other light limiting conditions at another site (Steinman and Mulholland, 1995).

The complex interaction of benthic macro-invertebrates and fish within each lotic ecosystem (McCollum et al., 1998) suggest that comparing site systems may be difficult. The effects of periphyton growth are dependent upon the interaction of light, nutrient availability and grazing. Comparing algae bio-mass data from differing methods must be carefully assessed. Artificial substrate, as with natural substrate, have characteristics which may affect periphyton growth. Smoother substrates such as glass rods or slides may promote colonization of fewer taxa than rougher substrates such as the glass fiber filters or clay tile.

Benthic chlorophyll *a* data are effective for measuring algae bio-mass. However, excessive variability may exist because of the patchiness of periphyton distribution even under the best conditions. U. S. EPA recommended assessments be based on the mean of three or more replicate samples. Results of this study suggest eighteen replicates per treatment type were adequate, given a power criteria of 0.95, for testing comparisons. Testing for error type between each treatment comparison suggest there is less than a 5% chance of rejecting the null hypothesis when it is true (type I error), and /or failing to reject the null hypothesis when it is not true (type II error) (Appendix 4).

High chlorophyll *a* concentrations may indicate nutrient enrichment, while low concentrations may indicate low nutrient availability, toxicity, shading, grazing or other unknown variables. Chlorophyll *a* data should only be used in support of other data such as biological, chemical, and physical parameters.

Recommendations

Ecosystem parameters of interest to the investigator must be clearly identified and isolated by experimental design prior to implementing ecological monitoring. Ecological monitoring include chemical, physical, and biological parameters. Complex ecosystem functions such as primary productivity must be measured with great care and high replication. The high variability and complexity of systems require high statistical replication in order to make meaningful comparisons.

Minimum number of Matlock Periphytometer replicates were calculated (α=0.05) using a confidence interval of 0.66 µg/cm^2 and a standard deviation of 0.42 µg/cm^2 (Appendix 4). Results indicated 10 replicates were required. Future testing should consist of floating periphytometers with screening confined to the bottom of the periphytometer rack to minimize light limitations. The periphytometers should be positioned away from the shorelines in deeper water with minimal tree canopy to reduce effects from sediment mixing and shading along the shallow bank areas. In addition, light meters should be placed on the periphytometers to monitor photo period and light intensity. Periphyton samples should be split to include taxonomic examination to assess select colonization limitations and document periphytic response.

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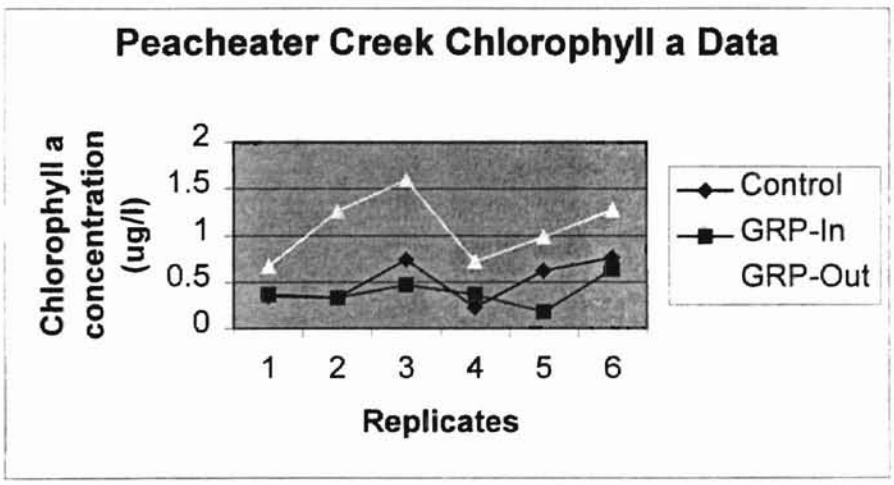
APPENDIX 1

CHLOROPHYLL a CONCENTRATIONS FOR PEACHEATER CREEK

DATA FOR CHAPTER 3

Table A – 1: Chlorophyll a data ($\mu g/cm^{-2}$) collected from Peacheater Creek between April 8 – 21, 1995.

Replicate Identification	Treatments Control	GRP – I	GRP – O
1	0.34	0.37	0.67
2	0.33	0.33	1.26
3	0.74	0.47	1.59
4	0.23	0.37	0.72
5	0.62	0.18	0.98
6	0.76	0.64	1.27



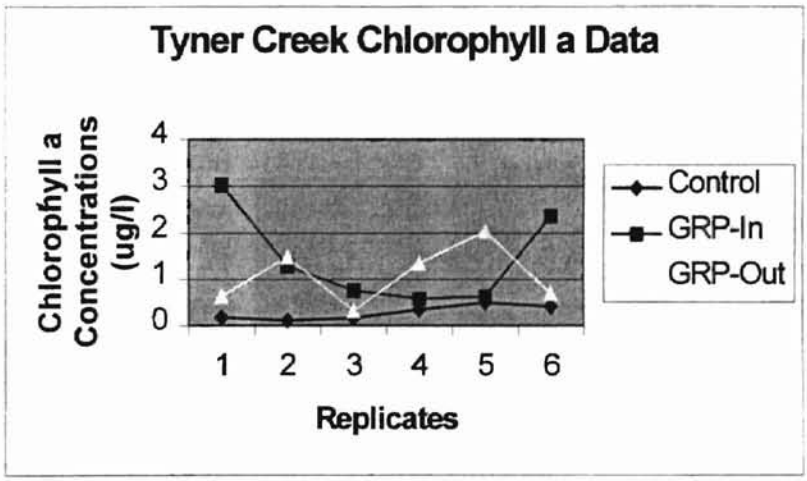
APPENDIX 2

CHLOROPHYLL a CONCENTRATIONS FOR TYNER CREEK

DATA FOR CHAPTER 3

Table A – 2: Chlorophyll a data ($\mu g/cm^{-2}$) collected from Tyner Creek between April 8 – 21, 1995.

Replicate Identification	Treatments Control	GRP – I	GRP – O
1	0.18	3.03	0.63
2	0.11	1.29	1.48
3	0.17	0.76	0.32
4	0.34	0.57	1.33
5	0.05	0.64	2.03
6	0.41	2.37	0.69



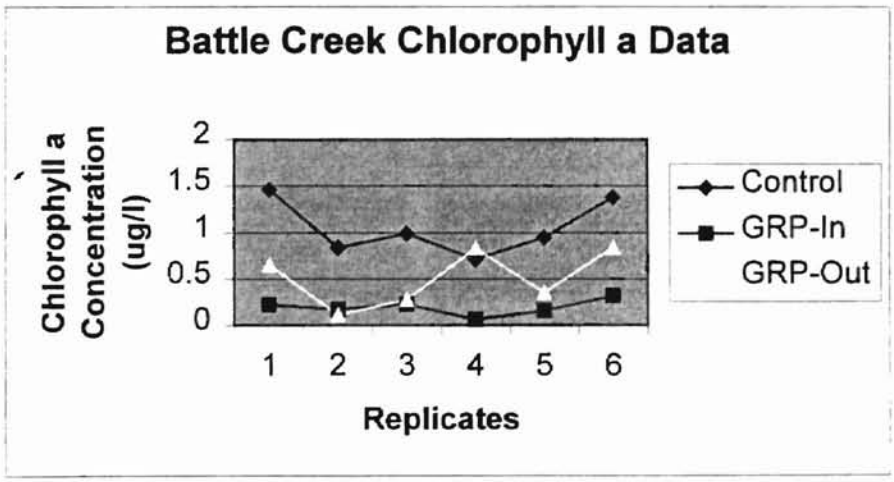
APPENDIX 3

CHLOROPHYLL a CONCENTRATIONS FOR BATTLE CREEK

DATA FOR CHAPTER 3

Table A – 3: Chlorophyll a data ($\mu g/cm^{-2}$) collected from Battle Creek between April 8 – 21, 1995.

Replicate Identification	Treatments Control	GRP – I	GRP – O
1	1.46	0.22	0.65
2	0.84	0.17	0.11
3	0.99	0.23	0.28
4	0.71	0.06	0.83
5	0.95	0.16	0.35
6	1.37	0.32	0.84



APPENDIX 4

REPLICATE NUMBER CALCULATION DATA

Equation (Steel and Torrie, 1996; equation 5.35):

$n = t^2s^2 / d^2$

where:

n = replicate number

t = t value, n-1 degrees of freedom, α=0.05

s = standard deviation

d = half-width of desired confidence interval

	MP-I	GRP-I	GRP-O
Mean (ug/cm^2)	0.59	0.42	0.89
Standard Deviation (ug/cm^2)	0.42	0.31	0.52

n = 10

t = 2.44

s = 0.49

d = 0.33

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